PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Licorice-induced hypokalemia in patients treated with Yokukansan preparations—identification of the risk factors in a retrospective cohort study
AUTHORS	Shimada, Saori; Arai, Tetsuaki; Tamaoka, Akira; Homma, Masato

VERSION 1 - REVIEW

REVIEWER	Takehiro Nakamura, MD, PhD Kagawa Prefectural University of Health Sciences, Japan
REVIEW RETURNED	15-Sep-2016

GENERAL COMMENTS	Reviewer's comment on bmjopen-2016-014218: Licorice-induced pseudoaldsteronism in patients treated with Yokukansan preparations –indication of risk factors for hypokalemia- In the present study the authors investigated pseudoaldsteronism, especially hypokalemia in patients treated with Yokukansan and Yokukansan-ka-chinpihange including licorice (glycyrrhiza radix) by retrospective study. Yokukansan could be commonly used for treatment of BPSD (behavioral and psychological symptoms of dementia) among dementia patients. The present article is interesting and shows very important information for clinicians. Overall, it could be improved if the authors might address the following: Here are several suggestions: 1) In discussion part, the first paragraph includes very important information. The authors should also show list of any Kampo medicines containing licorice and their ccontents (ex. 1.5 g/day) as a new Table. 2) There are very useful information in discussion part. If possible, the authors could make discussion part broken into section subheadings to be more systematic, due to easily understand.

REVIEWER	Eiseki Usami Department of Pharmacy Ogaki Municipal Hospital ,Japan.
REVIEW RETURNED	28-Sep-2016

GENERAL COMMENTS	・P2 L20 , P6 L20 Why did YK become a risk factor of hypokalemia in patients who were treated with YK? It may mean that YK became risk factor compared with YKCH? Is that indicated YKCH is better than YK to avoid hypokalemia? This result makes interpretation confusing. Please exclude "YK administration" as the risk factor of

	hypokalemia. ・Fig1 You should be analyzed in each of the items (age, body weight, YK (full dose, dosing period), co-administration of LPIDs, serum potassium, ALT, AST, ALB, BUN, Cre, etc.) of Table 2. Each risk factor should be analyzed by univariate logistic regression analysis. Age, albumin etc. should be analyzed by continuous variables and calculated cut-off values. Subsequently, the data should be analyzed using multivariate logistic regression analysis. ・Please write P-values in another column of Table2, 3. ・P9 L8 I wasn't able to understand hypokalemia onset days from figure 2. Please change from "Figure 2" to "Table 2". ・P9 L13 I wasn't able to identify "onset time by 15days" from figure 2. Can you do it? Please delete "(Figure 2)" of P9 L13. ・P9 L17 Why the wet extraction rate of GL is different even if the licorice contents are the same (1.5 g/day) in YK and YKCH? Is there any evidence? Please add references.

REVIEWER	Kenji Watanabe Keio University Japan
REVIEW RETURNED	03-Oct-2016

GENERAL COMMENTS	This article revealed the risk of YK and YKCH for pseudoaldosteronism. Although it is a retrospective study, this article is expected to open for the future prospective study. Authors should clarify several points. Major points: 1. Title is "Licorice-induced pseudoaldosteronism in patients treated with Yokukansan preparations". Without measuring renin and aldosterone level, it is hard to say pseudo aldosteronism. 2. Authors definition of hypokalemia is when potassium level became under normal range. Serum potassium level is affected by many factors including food intake. Is the change of potassium level from 3.7 to 3.5 same as 4.0 to 2.8? Please consider the definition of "hypokalemia". 3. Is the drop level of serum potassium independent from dose of YK preparations? 4. In Table 3, authors categorized the AST or ALT into normal group or abnormal group. In the abnormal group, is low serum AST or ALT included? 5. In Table 4, please separate co-medications from symptoms. 6. In Fig. 2, some patients reached hypokalemia after 1600 days from the start of YK preparations. Even 200 days from start of YK, is it really by YK preparations? If you cut 100 days from the start of YK preparations, the result will be totally different. Minor points. 1. Is the licorice in the YK preparations G. glabra and not G. uralensis? 2. Fig. 1 legend; should be changed to "a: patients with co-administraition of LPIDs. b: patients without co-administration of LPIDs"

REVIEWER	Francesco Sera Research Fellow in Medical Statistics Department of Social and Environmental Health Research, London School of Hygiene and Tropical Medicine London, WC1, UK
REVIEW RETURNED	07-Dec-2016

GENERAL COMMENTS	In this observational study the authors aim to evaluate the rates of hypokalemia in patients treated with licorice-containing Japanese traditional Kampo medicines Yokukansan (YK) and Yokukansan-ka-chinpihange (YKCH). The authors also evaluate risk factors that could modulate the rates of hypokalemia. The authors described this observational study as a retrospective case-control study and used a logistic regression model to analyse the data. In my opinion this study design could be analysed as a retrospective cohort study. In fact, it seems that the researchers have information on the time (days) on which the event (hypokalemia) occur or when the treatment was ended. It is not clear if they were able to record the last follow up time. There will be several advantage if data from this study will be analysed using time-to-event methods like Kaplan-Meier and Cox regression models; some of them are reported below; 1) In the abstract (page 2 line 12) the author claim that the main outcome is to measure the occurrence rate of hypokalemia. A rate by definition need to be referred to a given time period. In fact, it is not clear what time dimension has the estimates (24%) reported in the result section (page 6 line 8). The authors reported 34 days and the range (1-1600) days. Does the 24% estimate refer to the 34 days' period? I recommend to use methods (e.g Kaplan-Meier) that allows to estimate cumulative rates over a specific time period. 2) The logistic model used by the authors could give biased results on analysis time-to event data when the rate is not low and the follow up time increase over time. Moreover, the Odds Ratio deviate from the Risk Ratio when the event of interest is not rare. For example, in the discussion section (page 9 line 10) the authors state "Patients co-administered with LPIDs were 3.3 times more likely to develop to hypokalemia than with YK preparation alone", but the risk ratios as measured by the hazard ratios will be lower. I recommend using time-to-event methods (e.g Cox proportional hazard model). In these models each event is compared with controls at the time on which the event occurs. 3) Using time-to-event models is possible to have more information on some aspect of the treatment (e.g cumulative dose). These exposures could be modelled as time-varying covariates. Other points 1) Patient and study design section (page 5 line 18). Non compliant patient were excluded from the study. Could this choice bias the results: e.g. non compliant patient could have a more severe phenotype? 2) Patient and study design section (page 5 line 20). This sentence is not clear to me. Do the authors recorded laboratory data only when they observed a change? How a change was defined? 3) I think that the Odds Ratios presented in figure 1 are mutually adjusted in a multivariable logistic regression model. This procedure should be discussed in more detail in the Statistical method section (page 5 line 1-5).

	4) Have the authors considered in the multivariable logistic model to adjust also for the baseline potassium level? 5) To assess the effects of LPID co-administration on occurrence of hypokalemia the authors compared the period of treatment before the occurrence in the groups with and without LPIDs (figure 2). The author claim that a difference was observed (page 7 line 1-2), but not statistical test was performed or at least reported. I would highlight here that this analysis is exactly an example of time-to-event analysis (in fact these are Kaplan-Meier curves), and this analysis can be done for any other risk factors. Please note also that this analysis is analogous to estimate the association using the Odds Ratios in a univariate logistic regression, with the inflation of the Odds Ratio as stated before. 6) To eliminate the effects of LPID co-administration on the occurrence of hypokalemia the authors restrict the analysis on 302 patients treated without LPIDs (In page 7 line 3). The adjusted Odds Ratios presented in figure 1 (a) have the same interpretation: Odds Ratios for the other risk factors are “adjusted” for co-treatment under the hypothesis that the effect is the same in the groups with and without LPIDs. The subgroup analysis would adjunct a new set of information if there is an interaction between risk factors and co-treatment. Have the authors tested the interaction between risk factors and co-treatment?

VERSION 1 – AUTHOR RESPONSE

Responses to the comments of Reviewer #1

1) According to the suggestions, Table showing Kampo-medicines containing licorice were newly prepared as the Table 2 in the revised version.

2) According to the suggestions, discussion part was divided into 3 subheads parts, Occurrence rate of hypokalemia in Kampo-medicines, Hypokalemia in pseudoaldosteronism, and Risk factors for YK preparations-induced hypokalemia.

Responses to the comments of Reviewer #2

・P2 L20 , P6 L20:

Response:

Although licorice contents for both YK and YKCH are equal (1.5g/day), the occurrence of hypokalemia in YK (26.6%) was unexpectedly high compared with YKCH (12.1%). This means that YK has a higher risk factor for hypokalemia compared with YKCH as reviewer’s interpretation. This explanation was added in the results section (P6, L16-21) as easy to understand. A possible reason is the difference in GL contents between YK and YKCH as discussed in P10, L7-10.

・Fig1:

Response:

According to the suggestion, risk candidates were analyzed by Cox proportional hazard model including univariate and multivariate analysis as shown in Table 4 of revised version instead of Figure. 1 in original version.

・Please write P-values in another column of Table2, 3.

Response:

According to the suggestion, P-values were added in Table 3 of revised version (Table 2 in the original version). Table 3 in original version was deleted from the manuscript.

・P9 L8:

・P9 L13:

Response:

Figure 2 was revised by using Kaplan-Meier method according to the other reviewer's suggestion. Patients treated with concomitant LPIDs showed a shorter time-to-occurence for hypokalemia than those without concomitant LIPDs as shown in Figure 2. This result was statistically significant ($p<0.001$).

・P9 L17:

Response:

According to the suggestion, we added a reference reporting the GL extraction efficiency which was varied pH dependently in the decoction (Ref.28) (P10, L9).

Responses to the comments of Reviewer #3

1. According to the suggestion, Table 1 was corrected as the product information.

2. According to the suggestion, the description of YK preparations was indicated accurately throughout the text (P5, L3-9) and Table 1 in the revised version.

3. According to the suggestion, Table 1 was corrected as indicating the crude drugs.

4. According to the suggestion, "Glycyrrhiza glabra" with Italic was corrected to "Glycyrrhiza" throughout the text.

5. According to the suggestion, "Glycyrrhiza glabra" with Italic was corrected to "Glycyrrhiza" throughout the text.

6. The word of "compounds" was deleted.

7. According to the suggestion, this sentence was corrected in the revised version (P5,L4).

8. According to the suggestion, this sentence was corrected in the revised version (P5,L8, P8,L7).

9. According to the suggestion, "lyophilization" was corrected to "spray-drying" in the revised version (p10,L4,6).

Responses to the comments of Reviewer #4

Major points:

1. According to the suggestion, "pseudoaldosteronism" was corrected to "hypokalemia" in the title.

2. We understand this opinion. The potassium levels before administration of YK preparation is important for assessing hypokalemia. We, therefore, excluded the patients with potassium levels less than 3.6 mEq/L before administration of YK preparation from the study. When using the present criteria, 3.6 mEq/L, under taking YK preparation, potassium reduction (delta potassium) was significantly different between hypokalemia and non-hypokalemia (-0.7 vs. -0.1) even though the baseline potassium was lower in hypokalemia (4.0 vs. 4.2). We considered that the criteria could be used to discriminate hypokalemia from non-hypokalemia in retrospective observation. We, therefore, added data for delta potassium in Table 3 of revised version.

3. We compared the data of delta potassium between full dose and reduced dose of YK preparations.

Although higher reduction in full dose was observed, difference was not statistically significant. We, therefore, did not describe the difference in the text.

4. Table 3 of original version included patients with low serum ALT as the laboratory abnormality. According to the suggestion, we excluded the data from the Table 3 of revised version.

5. According to the suggestion, co-medications are separated from symptoms in Table 5 of revised version (Table 4 in original version).

6. Because a case of pseudoaldosteronism that was developed after 3-year (over 1000 days) administration of tokishigyakukagoshuyushokyoto (licorice contents: 2.0g/day) was reported (Ref.27), we monitored serum potassium for over 200 days. This explanation was added in discussion section (P9,L19) in the revised version.

Minor points.
1. Licorice in the YK preparation is G. uralensis. According to the suggestion of reviewer 3, “Glycyrrhiza glabra” was corrected to “Glycyrrhiza” in the revised version.

2.We changed Figure 1 in original version to Table 4 in revised version.

Responses to the comments of Reviewer #5
1) According to the referee’s suggestion, the data for occurrence rate of hypokalemia was re-analyzed by Kaplan-Meier methods (Figure 1). Cumulative rate of hypokalemia described in results section (P6,L14-15) in the revised version.

2) According to the referee’s recommendation, we used Cox proportional hazard model based on univariable and multivariable analysis to determine the hazard ratio s of independent factors for hypokalemia (Table 4) in the revised version.

3) According to the referee’s suggestion, we tried to analyze the cumulative dose as the candidate of risk factor. But we did not determine it as the significant risk factor for hypokalemia. We, therefore, did not discuss this issue in the text.

Other points
1) As referee pointed out, dugs possessing severe adverse events like anti-cancer agents provide a cause of non-compliance resulting in bias the results. Present case, like Kampo-medicines that are generally safe drugs, may not affect the compliance due to the adverse events. We, therefore, excluded noncompliant patients from the study to assess the licorice-induced hypokalemia.

2) Because the present study is retrospective observational one, we collected the data from the medical records. We change the sentence to be clear (P5,L21,24) in the revised version.

3) According to the suggestion, Figure 1 in original version was reconstructed to Table 4 by using Cox hazard model in revised version and the detail of statistical method was added to the text (P6, L4-8).

4) We really appreciate your comment for this matter because we could find our mistake on analyzing the data. We have not considered in the multivariable logistic model to adjust for the baseline potassium level because of luck of significance. But, this was mistake. We re-analyzed the data and found that the difference between the two groups was statistically significant. We, therefore, included baseline potassium for multivariable analysis on Cox hazard model as shown in Table 4 of revised version.

5) According to the suggestion, we analyzed the effects of LPIDs co-administration on occurrence of hypokalemia by Kaplan-Meier methods and the significant difference was observed (Figure 2).

6) We completely agree with this comment. We, therefore, decided to remove Table 3 and Figure 1of original version from the manuscript and newly constructed Table 4 to describe the risk factors for hypokalemia in revised version.

VERSION 2 – REVIEW

REVIEWER	Takehiro Nakamura, M.D., Ph.D. Kagawa Prefectural University of Health Sciences, Japan
REVIEW RETURNED	31-Jan-2017

GENERAL COMMENTS	I have no more comments.

REVIEWER	Eiseki Usami Department of Pharmacy, Ogaki Municipal Hospital, Japan
REVIEW RETURNED	31-Jan-2017

GENERAL COMMENTS	This will be one of the guidance for YK-administered patients.

REVIEWER	Francesco Sera Department of Social and Environmental Health Research, London School of Hygiene and Tropical Medicine, London, United Kingdom
REVIEW RETURNED	13-Feb-2017

GENERAL COMMENTS	I think that the manuscript has improved from previous submission. The authors have answered to all my comments. In particular a correct time-to-event analysis was used to analysed the retrospectively collected cohort data. I have just few minor comments: 1. Table 4. I think two decimal digits are enough to describe hazard ratios and 95%CI. 2. I'm not a native English speaker, but it seems to be that it would be better to revise the English before final submission.

VERSION 2 – AUTHOR RESPONSE

- Your response to comment 5 from reviewer 3 is identical to comment 4 (namely: “According to text.”). Was this a mistake? Please clarify. The relevant reviewer comment is: “Japanese Pharmacopoeia defines “Glycyrrhiza” as “the root and stolon, with (unpeeled) or without (peeled) the periderm, of *Glycyrrhiza uralensis* Fischer or *Glycyrrhiza glabra* Linné (Leguminosae). Which plant does the TSUMURA use? In usual, Kampo medicine prefers to use the root of *Glycyrrhiza uralensis* .”

Response:
Reseponse for the comment 4 and 5 from reviewer 3 is same (not mistake). We supposed to explain this issue in detail in previous letter. We tried to confirm which plant is used, Glycyrrhiza uralensis or Glycyrrhiza glabra, for making YK preparation in Tsumura Co. The company, however, did not provide the clear information for this isuue. We, therefore, use Japanese Pharmacopoeia defines “Glycyrrhiza” as the crude drug name in stead of special plant name as shown in above comments.

Responses to the comments of Reviewer #5

1. Table 4. I think two decimal digits are enough to describe hazard ratios and 95%CI.

Response:
According to the suggestion, Table 4 was corrected.

2. I'm not a native English speaker, but it seems to be that it would be better to revise the English before final submission.

Response:
According to the suggestion, English was checked by native English speaker, again.